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13. ABSTRACT (Maximum 200 Words) We have generated 81 individual clones of the LNCaP C4-2 subline genetically tagged with bacterial β -galactosidase (BAG), and a separate C4-2-BAG-pool. The pool and 14 individual clones have been tested for PTHrP secretion <i>in vitro</i> and inoculated intra-cardially or subcutaneously into male SCID mice. BAG-PCR analysis showed a high degree of bone metastasis in 2 clones, medium in 4, low in 5, and none in 4. The BAG-pool, two high and three low bone-metastasising clones are being subjected to repeat characterization in the SCID mice with intracardiac, subcutaneous, and intra-prostatic inoculation. In each case, material will be kept for histological analysis, ISH and IHC of PTHrP, and Laser Capture Microscopy (LCM). LCM has been developed for the human clinical material from radical prostatectomies, and clinical material, including primary prostate cancers, metastatic lymph nodes, associated seminal vesicles, and bone metastasis material, has been extended. We will ultimately perform gene array analysis on clones with differential bone metastasis potential, as well as at different stages in the mouse model, and examine the expression of candidate genes in the accrued clinical material.				
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FOREWORD

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INTRODUCTION:

Although prostate and breast tumours both demonstrate a predilection for metastasis to bone, metastases to bone from prostate are predominantly osteoblastic while those from breast tumours are predominantly osteolytic. The molecular basis for this difference is not known, and may be exploited in controlling this very painful manifestation of prostate cancer. Also, it is likely that osteolytic activity, which often is present in prostate bone metastases, is actually required for the osteoblastic nature of prostate bone metastases. If so, inhibition of this process may be palliative in prostate tumours also. Our approach is to develop a genetically tagged series of clonal sublines from a well characterized prostate cancer cell line which will show differential bone metastatic potential in our intra-cardiac model. We will then generate a molecular profile of these sublines in order to identify genes associated with each stage of the bone metastasis process. These will be validated in clinical material which we are accruing, and will subject to laser capture microdissection.

BODY:

Task 1: To genetically tag the LNCaP-C4-2 cells, generate individual subclones, and determine the time course for bone metastasis. (Aim 1):

We originally proposed to employ the TSU-pr1 cell line, based on our previous observation of positive bone metastasis in that cell line. However, after submitting the application, we became aware of a series of sublines generated by *in vivo* selection of the LNCaP prostate cancer cell line. The LNCaP cell line was first isolated from the lymph node metastasis of a prostate carcinoma (1,2), and the cell line represents a well accepted model for androgen-dependent PrCa. Chung and colleagues have used co-inoculation with bone marrow stromal cells to develop LNCaP tumours in both intact and castrated SCID mice, and derived a series of sublines from these mice with different levels of androgen-dependence (3). In particular, the C4-2 sub-line causes paraplegia in 20-50% of animals, and gives rise to a low level of osteoblastic lesions characteristic of those seen in the majority of prostate cancer patients (4). Consistent with our original proposal, we have generated C4-2-BAG subclones during the genetic tagging process, which we have subsequently examined for metastatic potential in SCID mice. We chose C4-2 since it leads to a low level of bone metastasis which authentically resembles the human condition (4,5), such that derivation and comparison of both highly competent and poorly competent (for bone metastasis) clones should be possible. Although the time required for the metastasis of LNCaP may be longer than we anticipated with TSU-pr1 cells, the ultimate formation of such authentic metastasis made them the model of choice.

1.1 Lac-Z (BAG) Transduction and cloning: This has been successfully effected. The BAG viral supernatant was generated and used to infect the C4-2 cells obtained from UROCOR, USA. Eighty-one individual clones were derived, and the remaining G418-resistant cells were pooled to generate the C4-2-BAG-pool population. Each of the clones was expanded and frozen for storage. Fourteen of these have since been analyzed *in vivo* (see 2.2 below).

1.2 Pilot Study for Chronology of Bone Metastasis Ontogeny: This has not been initiated because of the lower frequency and longer duration required for osteoblastic lesions from the C4-2 cells (4). We felt that the incidence of bone metastasis from the parental C4-2 cells, as represented by the C4-2-BAG pool, would be too low, such that the experiment would be

Table 1: Summary of the features of C4-2-BAG clones tested to date.

Clone ID	Cell lines in vitro		Xenografts			I.C. inoculation
	PTHrP (pM)	Growth rate in vitro (unit absorbance/day)	Growth rate in vivo (mm ³ /day)	PTHrP IHC score	PSA IHC score	Metastasis status
C4-2	4.8	.07	513	3+	4+	
C4-2 BAG POOL	4.2	.14	309	3+	4+	Bone
1C3	<2	.14	-	NA	NA	Bone, MRT
1C3B	<2	.1	35	4+	3+	Bone
3F10	<2	.08	-	NA	NA	Bone, MRT, testes
3H5	2.3	.18	152	4+	4+	Bone, liver
1E2B	2.6	.09	47	4+	+	ND
3H3	2.7	.13	34	3+	4+	None
2B6	3.1	.09	91	3+	3+	None
1G2	3.2	.13	141	3+	3+	Bone
3C6	3.3	.1	130	4+	3+	Bone
3F9	3.5	.1	93	3+	+	Bone
3A6	3.6	.09	-	NA	NA	None
1D2	4.8	.15	89	+	3+	Bone
1G2B	6.2	.05	55	+	+	None
3G10B	13.9	.1	168	3+	4+	Bone, kidney
3G11	ND	ND	27	3+	4+	Bone

Clones are ordered from top to bottom on the basis of their PTH-rP expression levels *in vitro*, and highlighted according to bone metastasis potential from Table 2

High ☐, Medium ☐, Low ☐, None ☐

PTHrP: concentration of PTHrP in 24 hr conditioned media (collected under serum-free conditions) as detected by a N-terminally directed RIA. ND: not done.

Growth rate in vitro: 2000 cells seeded in 96 well plate, and cell number determined daily. ND: not done.

Growth rate in vivo: 5x 10⁶ cells suspended in 200 µl Matrigel inoculated subcutaneously. Once palpable, tumor diameter was measured every second day, and tumor volume estimated. -: no tumor established

IHC: intensity and number of cells staining represented on a scale of - (no staining) through to 4+ (most of tumor staining intensely). NA: not applicable.

Metastasis status: Presence of cells in tissue as determined by PCR. MRT: male reproductive tract. ND: not done. Full results shown in Table 2.

For each clone, *in vitro* characteristics of PTHrP levels (determined by RIA) and growth rate is indicated. *In vivo* attributes associated with subcutaneous xenografts in SCID mice are indicated, including an indication of growth rate and an estimate of PTHrP and PSA levels indicated by immunohistochemistry (IHC). An indication of the relative metastasis sites, taken from Table 2, is given. Clones coloured yellow show pronounced metastasis to both long bones and spine, while those in blue are medium, green low, and pink none. Metastasis to other organs was not been fully analysed, but has been seen so far in clones of high, medium and low bone metastatic potential.

Table 2: Summary of the metastatic profile of C4-2-BAG clones tested to date.

	2B6	3G11	1C3	1C3B	1D2	3F10	1G2B	3A6	3F9	3H5	3C6	3G10B	1G2	3H3	C4-2BP
Bone	0/7	1/6	2/8	1/8	0/4	3/6	0/7	0/8	5/8	7/9	1/7	0/8	2/7	0/7	2/9
MRT	0/7	0/6	1/8	ND	0/4	2/6	0/5	N/D	0/8	NC	0/7NC	0/8	ND	ND	0/5
Testes	ND	ND	0/8	ND	0/4	1/6	0/5	N/D	0/8	0/3NC	0/7NC	0/8	ND	ND	ND
Liver	ND	ND	ND	ND	ND	ND	ND	N/D	0/6	3/7	ND	ND	ND	ND	ND
Spleen	ND	ND	ND	ND	0/4	ND	0/7	N/D	0/8	0/8	0/7NC	0/8	ND	ND	0/3NC
Heart	ND	ND	ND	ND	0/4	ND	0/7	N/D	0/7	0/8	0/7	0/8NC	ND	ND	0/2NC
Brain	ND	ND	ND	ND	0/4	ND	0/7	N/D	0/8	0/8	0/7	0/8NC	ND	ND	0/3NC
Kidney	ND	ND	ND	ND	0/4	ND	0/5	N/D	0/8	0/8	0/7NC	1/8NC	ND	ND	0/6
Lung	0/7	ND	ND	ND	0/4	0/6	0/7	N/D	0/8	0/8	0/7	0/8	ND	ND	0/2
Vertebrae	0/7	0/6	0/8	2/8	1/4	0/6	0/7	0/8	3/8	1/8	2/7	1/8	1/6	0/8	0/7

Clones are highlighted according to bone met potential : High ☐, Medium ☐, Low ☐, None ☐

MRT = male reproductive tract

ND = No PCR has been done

NC = Only 1 PCR carried out

Total mice may vary in some cases, this is because PCR has not been carried out on all the samples.

wasteful. Instead, we will perform the careful time course analysis in a mixture of the competent clones, or with individual competent clones, depending on the number we generate (currently 2/15). Once the time course is established, we will obtain stage specific RNA samples by LCM as originally proposed. We will also then test up to 10 non-competent clones which show strong growth intra-prostatically and subcutaneously to determine whether they arrest at one of the three different stages designated in the application (i) adherence to the bone endothelium (ii) micrometastasis or (iii) macrometastasis. If necessary, we will also employ direct intra-tibial injection of the non-competent clones to determine whether they indeed can be supported by the bone environment. Thus, this aim is slightly modified in accordance with this more representative C4-2-BAG model, and will be carried out in year 2 of the grant.

Task 2: To determine interrelationships between organ- and bone-metastatic potential and PTHrP expression among the TSU-pr1-BAG clones. (Aim 2)

2.1 PTHrP Analysis of clones: We were not able to perform PTHrP analysis on each clone as it was generated, as initially projected, due to logistical difficulties of handling up to 80 clones at the same time combined with complications of comparative analysis of clones at different times. Instead, we are randomly selecting clones for *in vivo* testing, and measuring PTHrP levels at that time. Secreted PTHrP has been analysed by RIA for the 15 clones tested so far *in vivo*, and is summarized in Table 1 above. We have not yet performed the Western analysis with region specific antibodies to detect any altered processing, as we are waiting to do this on xenograft versus cell culture for each clone, and the same applies for the analysis of possible alternative splicing of PTHrP by Northern analysis. These will be done in the early part of year 2. PTHrP levels in the subcutaneous xenograft has been determined by immunohistochemistry, as shown in Table 1.

2.2 In Vivo Analysis of TSU-pr1-BAG clones: As summarized above, and shown in Table 1, 14 C4-2-BAG clones have been tested so far for growth analysis *in vitro* and *in vivo* (subcutaneous). In addition, each of these has been inoculated intra-cardially into male SCID mice to assess competence for bone metastasis. 12 of the 14 showed strong subcutaneous growth, 2 showed strong bone metastasis, 4 medium level of bone metastasis, 5 low levels, and 3 were distinctly negative for bone metastasis, despite three of them showing good subcutaneous growth. We have chosen 3 negative and 2 strong bone-positive clones for re-evaluation, and these will then be subjected to an extended repeat analysis involving intra-prostatic inoculation, subcutaneous inoculation, and intra-cardiac inoculation. These will be followed by time course analysis, and will provide the material for Aim 3, to be initiated at Month 19, as per the SOW.

Task 4: Accrual and Extended analysis of clinical material (Aim 4)

4.1 Identification and scheduling of candidate cases: We have continued the monthly meetings between Drs. Costello (urologist; primary prostatectomy), Choong (orthopedic surgeon, bone metastases) and Slavin (Pathologist) and this has proven very effective in facilitating accrual. As shown in Table 3, our current accrual has generated **115 biopsies, 40 primary tumours, xyz...** If projected over the second year of the grant, and will be well placed to initiate the Phase II gene expression analyses.

Table 3: Summary of tissue accrual to date.

	Biopsy	Sem Ves. Rt lobe Lft Lobe	LN	Biopsy & Primary (matched)	Bone metastasis
accrued before study		9			
Accrued in first year		32	2		2
current totals	112	41	2	10	2

4.2 LCM of clinical material at different prostate CA stages:

To date, specimens have been harvested by Dr Slavin in Pathology directly into liquid nitrogen, and are catalogued and stored at minus 70°C. We have developed the conditions for laser capture microscopy followed by RNA extraction, and have applied these to one of our frozen prostate carcinoma specimens, as shown in Figure 1. Our strategy is to accumulate these specimens over the next 6-12 months whilst we concentrate on Aims 1-3, so as to take best advantage of any new developments in the LCM technology, before we apply this. However, we have determined that our methodology is adequate to generate viable RNA from the frozen tumour specimens after LCM.

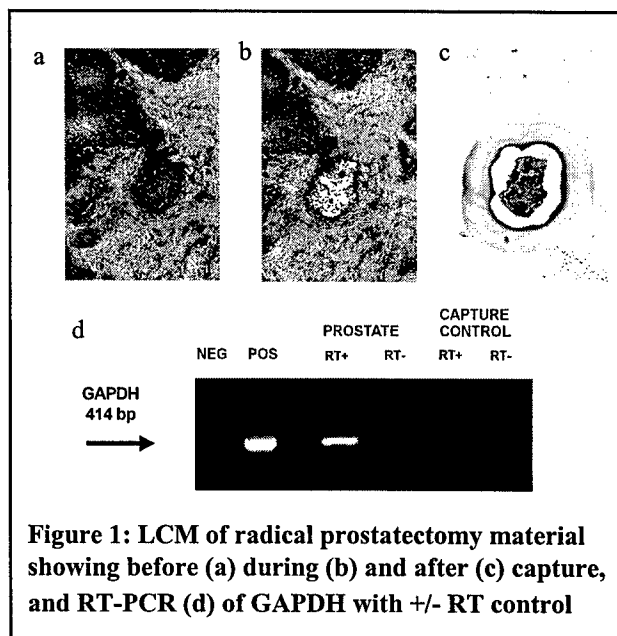


Figure 1: LCM of radical prostatectomy material showing before (a) during (b) and after (c) capture, and RT-PCR (d) of GAPDH with +/- RT control

KEY RESEARCH ACCOMPLISHMENTS:

- Established successful genetic tagging of a human prostatic cell line with proven osteoblastic bone metastasis capacity
- Screened sufficient clones for bone metastasis to generate a subset for comparative analysis of gene expression
- Tentatively determined a lack of relationship between PTHrP and bone metastatic potential of these clones
- Established a prostatic tumour tissue repository amenable to laser capture microscopy (LCM) and RNA extraction
- Effected successful LCM and RNA extraction for accrued frozen prostatic carcinoma tissue

REPORTABLE OUTCOMES:

Abstracts: We have generated one abstract outlining the approach for an International metastasis meeting (Appendix #1), as well as a comparative analysis of the C4-2 cells with other prostatic cell lines for aspects of basement membrane invasiveness common to human breast cancer progression (Abstract #2). The gene array analysis to be employed in Phase II has been applied to a parallel project in molecular pharmacogenetics of prostate cancer chemoprevention in humans, for which Dr. Williams undertook a UICC-funded training fellowship with the Advanced Technologies Center, NIH. This work relates to chemoprevention studies of Co-Investigator Costello (Urologist), and has led to an abstract which was technically supported in part by the DOD funding. During this time, Dr. Williams was replaced by Dr. Javni for continuity of the clonal analyses, as indicated in the authorship of Appendix 1.

Cell Lines: Once fully characterized, we will make our BAG-tagged C4-2 clones, available to other scientists, subject to approval by Urocor, from whom we purchased the cells.

CONCLUSIONS:

The LNCaP-C4-2 subline is the only cell line reported to generate osteoblastic lesions in any model anywhere. The strike rate reported was 20%, and we have already generated clonal, genetically tagged sublines which showed 100% metastasis to bone. Once confirmed, this will provide a valuable resource for ourselves and others to characterize the sequence of molecular events leading prostate bone metastasis. Our goal, which is the molecular profiling in prostate cancer of different stages of bone metastasis, will also be possible using the bone metastasis-competent clones we have already developed as well as sibling clones which we anticipate will be incompetent at different stages of metastasis. Molecular understanding of these mechanisms will enable better characterization of prostate tumours at the time of diagnosis, and may lead to palliative therapies which would limit bone colonization and the accompanying morbidity, in prostate cancer sufferers.

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APPENDICES:

1. Javni, J.A., Williams, E.D., Price, J.T. Waltham, M.C., Moseley, J.M., and Thompson, E.W.. Genetically tagged sublines of the LNCap variant C4-2 for molecular analysis of bone and soft organ metastasis in SCID mice. Beatson International Cancer Conference "Invasion and Metastasis", Glasgow, Scotland, June 27-30, 1999.
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Genetically tagged sublines of the LNCaP variant C4-2 for molecular analysis of bone and organ metastasis in SCID mice
J.A. Javni¹, E.D. Williams², J.T. Price¹, M.C. Waltham³, J.M. Moseley⁴ and E.W. Thompson^{1,3}

¹VBCRC Invasion and Metastasis Unit, ²St. Vincent's Institute of Medical Research, 41 Victoria Parade, Fitzroy, Melbourne, Victoria 3065, Australia and the Departments of ³Surgery and ⁴Medicine, The University of Melbourne.

The C4-2 human prostate carcinoma cell line (a subline of LNCaP), displays many features of advanced human prostate carcinoma in experimental *in vivo* murine systems. This includes androgen independence and the ability to metastasize to bone and lymph nodes following intracardiac, orthotopic or subcutaneous injection.

We have generated C4-2 human prostate carcinoma cells retrovirally transduced with the "BAG" expression vector that encodes both neomycin resistance and β -D-galactosidase. The former serves as a stable selection marker *in vitro*, while β -D-galactosidase functions as an aid to track prostate cancer metastasis in the *in vivo* models either by histological analysis and/or PCR. Single cell clones (designated C4-2 BAG) from the transduced pool were subsequently generated in order to screen and identify sublines with differing organ-targeting metastatic potential.

Initial analysis of parental C4-2 and C4-2 BAG clones indicated considerable disparity amongst sublines based upon the morphology of some of the clones *in vitro*. While the majority of the C4-2 BAG clones have a similar morphology to the parental C4-2 (stellate), others grow in clusters or are cobblestone in appearance. For *in vivo* analysis, SCID mice were injected intracardially with either parental C4-2 or individual C4-2 BAG clones. At four to six weeks post-injection mice were assessed for metastatic bone damage by X-ray (faxitron) analysis to distinguish lytic or sclerotic lesions. Harvested bones and soft tissues were analyzed by "BAG" PCR for evidence of metastatic spread. For additional comparison of sublines, cells were also grown, embedded in Matrigel, as a subcutaneous tumour and tumour growth rates monitored.

These well characterized C4-2 BAG clones should enable us to further define and understand the mechanism of metastatic spread of prostate cancer and the molecular basis associated with the preferential spread of prostate cancer cells to bone.



INVASION AND METASTASIS

Sunday June 27th – Wednesday June 30th 1999

Glasgow, Scotland

#1570 Evidence of epithelial-mesenchymal-transition (EMT) - mediated metastatic progression in human prostate carcinoma cell lines. Williams, E.D., Tester, A., Harrison, T., Moseley, J.M., Thompson, E.W., and Waltham, M. *VBCRC Invasion and Metastasis Unit, St. Vincent's Institute of Medical Research, and Departments of Medicine and Surgery, University of Melbourne, Fitzroy, Melbourne, 3065, Australia.*

Human breast cancer (HBC) cell lines with high metastatic potential in nude mice express the mesenchymal-associated intermediate filament protein vimentin (VIM), indicating the involvement of an epithelial-to-mesenchymal transition (EMT) in HBC progression. We analysed a series of human prostate carcinoma (HPrCa) cell lines for evidence of the EMT, and associated aggressive features. The metastatic PC-3, DU145, and TSU-Pr1 cell lines showed abundant expression of vimentin, and showed high levels of basement membrane invasion *in vitro* as seen with VIM-positive HBC cell lines. In contrast, the poorly metastatic, androgen-dependent LNCaP cell line lacked vimentin expression and showed poor Boyden chamber invasion. As with HBC cell lines, we further found associated VIM expression in the HPrCa cell lines with MMP-2 activation via the cell surface activator MT1-MMP, suggesting that this pathway may also mediate the invasive and metastatic potential of EMT-derived prostate carcinoma metastases. Interestingly, the LNCaP-derived C4, C4-2, and C4-2B sublines (UROCOR, Inc.), which show androgen-independence, enhanced tumorigenicity, and spontaneous metastasis in longer term studies, did not gain vimentin expression, indicating alternative metastatic pathways perhaps akin to those which facilitate invasion and metastasis in the MCF-7-derived LCC-sublines. Supported by VBCRC and US DOD DAMD17-98-1-8554.

#1571 Plasma versus serum prostate specific antigen values in advanced prostate cancer. Liu, Y.Q., Kyle, E., Lieberman, R., Bergan, R.C. *Northwestern University, Chicago, IL 60611 and National Cancer Institute, Bethesda, MD 20892.*

The use of serum Prostate Specific Antigen (PSA) is limited. As PSA is protein bound, we hypothesized that there may be important differences between plasma (pl) and serum (ser) PSA. Fifty-six paired pl and ser samples were collected from 14 patients with hormone refractory prostate. PSA standards were used to compare the accuracy of the Tandem-E sandwich ELISA PSA kit from Hybritech. PSA was measured in a batch fashion, and the pl/ser PSA ratio calculated as a percent of ser PSA. Average pl/ser ratios were then calculated for all PSAs in the range of 0-50, 51-100, 101-200 and 201-1000, and gave 92 ± 5.7 , 117 ± 5.7 , 125 ± 8.3 and 142 ± 6.0 , respectively. These findings demonstrate that there is an increase in the pl/ser PSA ratio with increases in overall PSA level. Plasma PSA should be further evaluated in a larger series, and in different patient populations, for potential prognostic significance.

#1572 Growth of heterotopic LNCaP cell tumor in nude mice is not affected by dietary calcium. Balaji, K.C., Fair, R.W., Huryk, R.F., and Heston, W.D.W., George M. O'Brien Urology Research Center, Memorial Sloan Kettering Cancer Center, New York, NY.

Some epidemiologic studies have suggested that increased calcium intake is significantly associated with advanced prostate cancer. We evaluated the role dietary calcium in this tumor system using nude mice models. The results at end of ten weeks is as follows:

Type of diet	Tumor weight (g)	Serum PSA (ng/ml)	PSA/tumor weight
Stress diet (high fat, 40 Kcal% and low calcium)	0.535 \pm 0.389, SE 0.087	125.4 \pm 87.9, SE 21.4	246.99
Stress + Ca ⁺⁺	0.578 \pm 0.435, SE 0.097	131.5 \pm 82.3, SE 20.07	242.714
Stress + VitD	0.555 \pm 0.463, SE 0.103	119.8 \pm 91.3, SE 23.71	316.878
Stress + Ca ⁺⁺ & VitD	0.495 \pm 0.332, SE 0.074	132.2 \pm 82.7, SE 19.46	272.943

Multiple pairwise comparison using analysis of variance showed no statistically significant difference between the various groups suggesting that dietary calcium does not affect LNCaP prostatic tumor growth in the nude mice model.

#1573 Basic fibroblast growth factor in spontaneous canine prostate cancer. Coffman, K.T., Ritmeester, A.M., Allen, D.K., and Waters, D.J. *Purdue University, West Lafayette, IN 47907.*

The dog is a spontaneous model to study factors that regulate prostate cancer progression. The pro-angiogenic peptide, basic fibroblast growth factor (bFGF), has been implicated in prostate cancer progression. To investigate the role of bFGF in prostate cancer, we used a commercially available ELISA test kit to quantitate bFGF in: conditioned media from 6 canine prostate carcinoma cell lines; urine from athymic mice bearing canine prostate carcinoma xenografts; and urine from 18 dogs with spontaneous prostate carcinoma. Three canine prostate cancer cell lines released low levels (0.3-1.2 pg/ml) of bFGF which were comparable to results for PC-3 and LNCaP human prostate cancer cells. In contrast,

three cell lines exported very high levels of bFGF into conditioned media (30-150 \times greater than in media conditioned by low bFGF exporting cell lines). Athymic mice bearing SQ tumors from the highest bFGF exporting cell line had urine levels 12 \times greater than median values for non-tumor bearing mice. Mice bearing SQ, intraprostatic, or intratibial tumors induced by low exporting cell lines showed no increase in urine bFGF. Dogs with prostate carcinoma had significantly greater urine bFGF levels than normal dogs ($p = 0.04$). Fifteen of 15 dogs with metastatic prostate cancer had elevated urine bFGF levels that ranged from 4 to 232 \times greater than the median value for normal dogs. Two of 3 dogs with organ-confined prostate carcinoma had normal urine levels. Our data further support the role of bFGF in prostate cancer progression. Canine prostate carcinomas do not express PSA, but urine bFGF may serve as a biomarker to assess treatment response in dogs with prostate carcinoma and in athymic mice bearing canine prostate carcinoma xenografts.

#1574 Hydroxyurea induction of micronuclei in prostate cell lines. Rivera, O.J. and McGill, J.R. *University of Texas Health Science Center at San Antonio, San Antonio, TX.*

Hydroxyurea (HU) is a chemotherapeutic compound which induces the formation of micronuclei in the cancer cell lines COLO320DM and HL60. We examined the effect of HU on micronuclei formation in prostate cell lines. Micronuclei bud off from the nucleus and are known to contain double minute chromosomes (dmns) or amplified genes. The micronuclei are then lost to the environment when the cell divides. Amplified genes and dmns are found in tumor cells and micronuclei formation can be used to develop methods to identify cancer-related gene sequences. In this study we exposed three prostate cancer cell lines (PC-3, DU-145, and LNCaP) to different concentrations of hydroxyurea (75 μ M, 150 μ M, and 200 μ M) and examined the effects on micronuclei formation after 14 and 21 days. We found that the percentage of micronuclei increased by 15.3% for PC-3, 2.93% for DU-145, and 4.6% for LNCaP as observed at day 21. We examined the micronuclei for the presence of centromeric DNA by FISH. Centromeric DNA was not found, indicating extrachromosomal elements were present. These results demonstrate that drug induction of micronuclei in prostate cells can be exploited. The micronuclei are likely to contain amplified extrachromosomal DNA, and the HU-induced packaging of such DNA into discrete micronuclei should facilitate further characterization of tumor related amplicons. (Supported in part by N.I.H. grant P30 CA54174).

CLINICAL RESEARCH 7: Bladder and Kidney Cancers

#1575 Cyclo-oxygenase-2 (COX) expression by immunoblot analysis is increased in frequency and amount in primary cancers of the lung and bladder compared with matched normal tissue. Sweeney, C.J., Marshall, M. S., Barnard, D.S., Marshall, S.J., Yip, M.T. *Indiana University, Indianapolis, IN 46202.*

To investigate the frequency of COX-2 expression in various cancers, immunoblot analysis was performed on specimens of lung adenocarcinoma (LAC), squamous cell lung cancer (SqCLC) and transitional cell carcinoma (TCC) of the bladder. Expression was compared with matched normal adjacent tissue (NAT) samples. Co-expression of bcl-2 with COX-2 was also evaluated. Equal amounts of protein were located and the level of COX-2 expression was quantified by densitometry and expressed as a percentage of the positive control. Expression was present in 19 of 26 LAC specimens versus 5 of 16 specimens of NAT ($p=0.01$). The mean percent optic density (%OD) in the LAC specimens was 40.6% with a median of 24% (Range: 0-189%, versus 3.0% 0-15.6%) for the NAT. 11 of 16 cancer specimens had greater than 2 fold increase in %OD compared with the corresponding NAT. 11 of 16 SqCLC positive versus 2 of 10 NAT ($p=0.04$), and the mean %OD in the 25.1% with a median of 8.5% (R: 0-178%), versus 2.9% and 0% (R: 0-178%) for the NAT. 6 of 10 cancers had greater than 2-fold increase in %OD compared with corresponding NAT. 12 of 16 TCC specimens were COX-2 positive with 2 of 8 NAT ($p=0.03$). The mean %OD in the TCC specimen was a median of 27.9% (R: 0-183%) compared with 13.7% and 0% (R: 0-183%) for the NAT. 5 of 9 cancer specimens had greater than 2 fold increase in %OD compared with corresponding NAT. There was no correlation between bcl-2 expression and COX-2 expression. (Sponsored by Smith Kline Beecham).

#1576 Expression of cyclooxygenase-2 in invasive transitional carcinoma of the urinary bladder in humans and dogs. Mohammed K.N., Koki, A.T., Snyder, P., Foster, R.S. and Knapp, D.W. *Purdue U Lafayette, IN 47907; Monsanto Life Sciences, Chesterfield, MO 6301 of Urology, Indiana University School of Medicine, Indianapolis, IN 46202.*

Non-Steroidal Anti-inflammatory Drugs (NSAIDs) have been associated with remission of spontaneous canine transitional cell carcinoma (TCC) of the urinary bladder (Knapp et al. 1994), a model of human invasive bladder cancer. NSAIDs inhibit cyclooxygenase (COX), the rate-limiting enzyme for prostaglandin synthesis. Prostaglandins are mediators of inflammation and regulators of epithelial cell growth, and COX inhibition likely plays a role in the antitumor effects of NSAIDs.

FILTER-BASED, PROSTATE-SPECIFIC cDNA MICROARRAY DEVELOPMENT, VALIDATION AND APPLICATION

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A cDNA microarray comprised of 5185 different cDNAs spotted onto nylon membrane filters was developed for prostate gene expression studies. The clones used for arraying were identified by cluster analysis of >35,000 prostate cDNA library-derived ESTs present in dbEST. Total RNA from two prostate and one melanoma cell lines were used to make radiolabeled probes for filter hybridisations. The absolute intensity of each individual cDNA spot was determined by densitometric scanning and evaluated using a bioinformatics package (P-SCAN) developed specifically for analysis of cDNA microarray experiments. Results show that 89% of the genes showed intensity levels above background levels using prostate cell probes, compared to only 28% for the melanoma cell probe. Replicate probe preparations yielded results with *r* values ranging from 0.90 to 0.93 and coefficient of variation ranging from 16% to 28%. Replicate hybridisations yielded correlations of 0.97 with a CV of 20%. We report the development and validation of a cDNA microarray system that is sensitive, reliable, and demonstrates a low degree of variability that will prove useful for quantitative gene expression analysis in prostate-derived cells and tissue. We are currently using these filters to assess the gene expression changes induced by the treatment of a prostate cancer cell line with the chemopreventative agent selenium.

E. Williams supported by an UICC ICRET Technology Transfer Fellowship and US DOD DMAD 17-98-1-8554

GENE ARRAY ANALYSIS OF SELENIUM EFFECTS ON
PC-3 HUMAN PROSTATE CANCER CELLS

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There is considerable evidence for a role for selenium in decreasing prostate cancer incidence. Although this has been historically attributed to its anti-oxidant properties, it has become recently established that supra-physiological selenium (above that required for sufficient expression of seleno-enzymes) also offers an anti-tumourigenic effect. We have used cDNA microarray analysis to identify genes regulated by selenium in the prostate cancer cell line PC-3.

Total RNA was extracted and radiolabeled probes generated. Filter cDNA microarrays were purchased from Research Genetics Inc. (GF211 & GF221). The absolute intensity of each individual cDNA spot was determined by densitometric scanning and evaluated using a bioinformatics package (P-SCAN) developed specifically for analysis of cDNA microarray experiments. Thirteen genes have been identified as up-regulated by selenium treatment, and twenty genes as down regulated. We are currently investigating the temporal nature of changes in expression of these genes in PC-3 cells by Northern analysis.

Gene array analysis has allowed the rapid identification of previously unknown selenium-responsive genes. Further work with these genes should enhance our understanding of the mechanism(s) behind decreased prostate cancer incidence associated with dietary selenium supplementation, and could thus improve our implementation of such strategies.



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REPLY TO
ATTENTION OF:

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17 Jun 02

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Encl

PHYLLIS M. PINEHART
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